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13. ABSTRACT *(Maximum 200 words)*

The leukocidins are bicomponent pore-forming toxins that are structurally related to staphylococcal alpha-hemolysin, a single component toxin. alpha-Hemolysin has many applications in the area of biomolecular materials. Therefore, leukocidins have now been characterized to expand the set of tools available for materials engineering. The approach taken in the AASERT project was to obtain genes for the two components of the toxin, to express them in *E. coli*, to characterize the heterologously expressed pores by bilayer recording, and to use molecular genetic technology to explore structure- function relationships in these proteins. The first three of the four objectives have been accomplished.

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REPORT TITLE: Molecular Genetic Approaches to Biomolecular Materials

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SUBMITTED FOR PUBLICATION TO (applicable only if report is manuscript):

Sincerely,

Hagan Bayley, Ph.D.
Professor

MOLECULAR GENETIC APPROACHES TO BIOMOLECULAR MATERIALS

FINAL PROGRESS REPORT

HAGAN BAYLEY

NOVEMBER 15, 2000

DAAG5597-0173

THE TEXAS A&M UNIVERSITY SYSTEM HEALTH SCIENCE CENTER

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FINAL REPORT

GRANT #: DAAG5597-0173

PRINCIPAL INVESTIGATOR: Hagan Bayley, PhD; AASERT student: George Miles

INSTITUTION: The Texas A&M University System Health Science Center

GRANT TITLE: The student was engaged in research under the ARO grant DAAG5597-0173: Molecular genetical approaches to biomolecular materials

AWARD PERIOD: June 1 1997 - May 31 2000

OBJECTIVE: The leukocidins are bicomponent pore-forming toxins that are structurally related to staphylococcal alpha-hemolysin, a single component toxin that has been studied in detail by the Bayley group. alpha-Hemolysin has many applications in the area of biomolecular materials. Therefore, leukocidins have been characterized to expand the set of tools available for materials engineering.

APPROACH: Staphylococcal LukF and LukS together form transmembrane pores in lipid bilayers. The approach taken in the AASERT project was to obtain genes for the two components of the toxin, to express them in E. coli, to characterize the heterologously expressed pores by bilayer recording, and to use molecular genetic technology to explore structure-function relationships in these proteins.

ACCOMPLISHMENTS: The first three of the four objectives have been accomplished and have written up as a paper for Biochemistry. The genes for LukF and LukS were amplified from Staphylococcus aureus genomic DNA, placed in expression vectors and sequenced. The genes were also constructed by using synthetic oligonucleotides as building blocks. The latter was done so that the sequence homology at the nucleotide level could be increased, while maintaining the original amino acid sequences. This will allow gene shuffling experiments to be performed. The LukF and LukS genes were expressed in E. coli cells or by in vitro translation in an E. coli S30 system. The products were purified either in native or His-tagged form. The electrical properties of various combinations of subunits were explored by single-channel recording. The LukF/ LukS pore is larger than that formed by alpha-hemolysin and has other distinct properties including ion selectivity and gating characteristics.

CONCLUSIONS & SIGNIFICANCE: The availability of milligram amounts of the leukocidins will be useful for membrane protein engineering studies that will increase the diversity

of pores that can be formed by alpha-hemolysin and its relatives.

PATENT INFORMATION: not applicable

AWARD INFORMATION: none

PUBLICATIONS:
Miles, G., Braha, O., Cheley, S. and Bayley, H. (2000) Biochemical and biophysical characterization of recombinant LukF/ LukS, a bicomponent pore-forming toxin, Biochemistry, submitted